Remarks

Applicants respectfully request reconsideration. Several of the claims are hereby amended in direct response to the claim rejections, and to address the grounds of the rejections. Applicants also gratefully note that the Examiner has withdrawn the restriction requirement with respect to election of species.

By this Amendment, Applicants are amending the previously presented claim 1 from a ***product*** claim for oligonucleotides into a ***method*** claim for assembling the oligonucleotides for preferentially killing cancerous cells over non-cancerous cells. This method of combining a prodrug antimetabolite with the oligonucleotide to assemble the preferred oligonucleotides is novel, and together with the disclosed linkage protocols forms the core of the invention.

The disclosed method does not claim a "genus" of oligonucleotide species. Furthermore, it is *same* regardless of the prodrug antimetabolite used, or the initial oligonucleotide to incorporate into the process of assembly. Therefore, applicability of method is not affected by the vastness of the number of potential oligonucleotide "species."

Further, the method of this invention presented in claim 1, does not suffer from the unpredictability of behavior normally associated with biomolecules.

Several of the claims that depend from claim 1 have been amended into method claims as well, and consequently, are similarly unaffected by either the large number of oligonucleotides or by the unpredictability of the behavior of biomolecules.

Any remaining "product" claims are narrowly drawn and fully supported by the disclosure of the patent application as filed.

Thus, claims 1-37 have either been amended as outlined above, or left unchanged in view of the withdrawal of species restriction by the Examiner.

Previously presented claims 38-44, directed to the non-elected invention, stand withdrawn as a result of the restriction requirement.

New claims 45-48, directed specifically to colon cancer have been added. Also new claims 49 and 50 have been added.

No new matter has been added by this Amendment.

Claim Rejections

35 USC 112

First Rejection under 35 U.S.C. 112, first paragraph

Claims 1-37 stand rejected under 35 U.S.C. 112, first paragraph.

The Office Action notes that claims encompass a genus of oligonucleotides, that the structure of the oligonucleotide comprising at least two CpG moeties is vast in view of the open claim language of "comprising", and that the structures of the additional nucleic acids in the oligonucleotides are not disclosed. [Pages 4 and 6 of the Office Action.]

The Office Action states further that because the claims encompass a genus of variant species, an adequate written description must include sufficient description of at least a representative number of species for a factual evidence of an actual reduction to practice. [Page 8].

The Applicants believe that by rewriting claim 1 as a ***method*** claim for assembling the oligonucleotides for preferentially killing cancerous cells over non-cancerous cells they have addressed these concerns.

For the method of the present invention, i.e., the method of combining a prodrug antimetabolite with the oligonucleotide(s), the *number* of oligonucleotides used in such a combination is of no consequence.

Furthermore, this invention discloses ***how*** a prodrug antimetabolite may be combined with the oligonuclotides for preferentially killing cancerous cells over non-

cancerous cells; it does not provide "functional specifications" of a biomolecule, i.e., antimetabolite that may be used for the purpose. Therefore, the issue of "insufficiency of a biomolecule sequence described only by a functional characteristic" does not arise for the amended method claims.

The Applicants are cognizant of the prior art, and agree with the recognition in the prior art of therapeutic potential of CpG ODN. Their invention builds on that recognition. By using the immunostimulatory property of CpG in combination with limited amount of potentially toxic antimetabolite prodrug, it teaches the way to optimize anticancer treatment. The Applicants provide compelling experimental data[1] in the case of the detailed working example of colon cancer, and GEMCITABINE as the drug of choice to preferentially kill these cancer cells.

While GEMCITABINE alone is known to cause ndiscrimite cytotoxicity and end up killing the patient and not the cancer because cancer cells always develop drug/multidrug resistance sooner or later, while the stable normal cells never do. The added advantage of selective killing of the genomically unstable cells is that it is not location-dependent. Thus even though the cancer cells have spread to distant corners of the body, the drug will seek them out and achieve differential killings.

The Applicants also show why the results could be duplicated for other cancers: The combination with CpG provides a powerful mechanism to deliver the antimetabolite precisely and selectively to the site of a cancer, but a guarded mechanism that tempers the toxicity of the prodrug and spares the noncancerous cells.

The reason for this optimism is available in the prior art. For example, Ballas et al.[2] have shown that CpG oligonucleotides with different sequences have divergent therapeutic and immunological effects. Although these authors initially theorized that single type of optimal CpG motif would work in all type of disease conditions or tumor types, it was found that different CpG motifs vary in their response towards activation of

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[1] Depicted in Figures 13 and 14.

[2] Ballas , Z.K.,Krieg, A.M., Warren, T., Rasmussen, W., Davis, H.L., Waldschmidt, M and Wagner, G.J., J. Immunol, 167, 4878, 4878-4886, 2001.

different cell types. Thus, for example, different ODNs trigger different responses such as the activation of B- cell, NK cells and dendritic cells, favor a Th1- like cytokine production. Similarly, a number of CpG oligonucleotides having different CpG motifs were found to have potent effect in inducing cytokine secretion , such as IL-6, IL-12, IFN-r and TFN-alpha, having ability to stimulate B cell proliferation and to induce NK cell killing activity.

Another discovery by Ekamber R. Kandimalla, Lakshmi Bhagat, Daqing Wang, Dong Yu, Fu-Gang Zhu, Jimmy Tang, Hui Wang, Ping Hwang, Ruiwen Zhang and Sudhir Agarwal, Nucleic Acids Research, 31: 2393-2400, 2003 reported potent immunostimulatory actiovity in CpR oligos and carried out a systematic study of CpR motif. The "R" in the CpG motif is essentially G component of CpG motif. The authors carried out modification in the "G" component, as represented by "R", and observed subtle differences in cytokine secretion profiles. In compounds where the structural modifications of R included modified nucleoside, 7-deaza-2'- deoxy guanosine. CpR immunomers induced higher interleukin (IL)-12 and lower IL-6 secretion, and showed p38 mitogen activated protein kinase pathway. The studies concluded that there is divergent synthetic nucleotide motif recognition pattern of receptor involved in immunostimulatory pathway and synthetic nucleotides can be developed to elicit different cytokine response patterns.

Several of the claims as amended concern the method of combining the CpG and prodrug into effective oligonucleotide products as well as the geometries of the linkages. The present invention does not get into the fray of numerous therapeutic agents that might be employed. Instead, it provides the place for a variable, generic sequence, and teaches how these other therapeutic agents may be incorporated in place of the generic sequence to customize the therapeutics for different cancers.

Thus, by amending claim 1 into a method claim, Applicants have addressed the concerns expressed in the Office Action of 2/26/08 leading to the first rejection under 35 U.S.C. 112, first paragraph,.

A second Rejection under 35 U.S.C. 112, first paragraph

Claims 36-37 are further rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement.

The concerns expressed in the Office Action relate to the following: The amount of experimentation required; the nature of "unpredictable arts such as chemistry and biology" in view of the prior art; the breadth of claims due to use of the term "pharmaceutical"; and, the silence in the disclosure of correlation between *in vitro* and *in vivo* success.

Applicants' respond to each of these concerns as follows.

The amount of experimentation required

Although there is the recognition in the prior art of the therapeutic potential of CpG ODN, the Applicants believe that they are the first inventors to propose the disclosed method, i.e., the method of combining a prodrug antimetabolite with an oligonucleotide having appropriate CpG moieties.

The Applicants have provided detailed experimental results for the case of colon cancer with the use of the antimetabolite GEMCITABINE; they have also provided extensive guidelines in the specification for using other antimetabolites. Armed with the

knowledge of specific antimetabolites that attack other types of cancer sites, practitioners can proceed with confidence of duplicating similar results for other types of cancers.

The reason for such confidence is the following generally applicable observation: The combination with CpG provides a powerful mechanism to deliver the antimetabolite precisely and selectively to the site of a cancer, but a guarded mechanism that tempers the toxicity of the prodrug and spares the noncancerous cells.

Therefore, knowing a suitable antimetabolite for a specific type of cancer only routine experimentation will be necessary to practice the invention.

The nature of "unpredictable arts"

Expectation of results for other forms of cancer

There exist art-recognized scientific reasons for the expectation that these results may be duplicated for other forms of cancer by strategically choosing the prodrug antimetabolite or utilizing a suitable oligonucleotide in the method of this invention for preferentially killing cancerous cells over non-cancerous cells.

The nature of the unpredictable arts such as chemistry and biology is not directly at issue in the practice of the method: once a suitable antimetabolite for a type of cancer is identified, the teachings of the present invention may be employed. The principle behind the method, i.e., CpG provides an effective mechanism to deliver the antimetabolite precisely and selectively to the site of a cancer but one that guards against the toxicity of the prodrug to spare the noncancerous cells, this principle provides an approach that is applicable in the case of other cancers.

The breadth of the claims

As to the breadth of the claims, the Applicants point to the language of amended claims 21, 22, 36 and 37. The amended claims are directed *only* to the pharmaceutical products made by the method claimed by the Applicants and therefore *narrowly* drawn.

The correlation between *in vitro* and *in vivo* success

Since the Applicants have amended the majority of the claims to method claims, the correlation between *in vitro* and *in vivo* success is not necessarily directly at issue. However, the inventors point to prior art that anticipates such correlation. In particular, the Applicants note the following:

(1) The CpG ODN, containing CpR motifs, which induced immunostimulatory activity in cell culture assay were generally found to have potent *in vivo* antimtumor activity.

(2) The role of CpG ODNs in cancer immunotherapy has been in the prior art, for example as reviewed elegantly in Carpentier[3] ;

(3) It was shown, for example, by Decker et al[4] that CpG ODNs have strong immunostimulatory effect in chronic lymphocytic leukemia B cells;

(4) It was concluded in the cited studies that CpG specific modulation is a key epigenetic mechanism in genomic imprinting, resisting nuclease restriction and patterning of chromatin conformations, and has powerful effect in modulating cell death.

(5) Since the house keeping genes are present in every cell and are responsible for essentials of cell function and survival , it is therefore possible to design a variety of sequences with a drug to enhance the effect of such CpG containing genes. This mechanism is elaborated below under the section heading "Further Discussion of the

[3] Carpentier, A.F., Auf, G. and Delattre, J.-Y Front. Biosci., 8, e 115-127, 2003.
[4] Decker, T. and Peschel , C. Leuk. Lymphoma, 42, 301-307, 2001.

Prior Art." That discussion includes in particular references to the work of Dr. Kwok-Hung Sit, one of the inventor Applicants of this invention.

Additionally, as noted above, the method of this invention provides the place for a variable, generic sequence, and teaches how these other therapeutic agents may be incorporated in place of the generic sequence to customize the therapeutic applications.

The importance of flanking sequences around CpG is very crucial and results in a specific biological activity, Krieg, A.M. etal., Nature, 374, 546, -549, 1995; Yammamoto, S. etal., Curr. Top. Microbial. Immunol. 247, 23-39, 2000. Bauer, S. etal., Proc. Natl. Acad. Sci., USA, 98, 9237-9242, 2001.

Therefore, in sum, the Applicants submit that the amended claims 21-22 and 36-37 address the above concerns expressed in the Office Action as to the second grounds of rejection under U.S.C. Section 112, First Paragraph.

Further Discussion of the Prior Art

and

Detailed experimental results by Applicants for colon cancer

The discussion below pertains to the prior art relied on in the Office Action.

Detailed experimentation was carried out by Applicants for colon cancer as disclosed in the specification as filed and summarized in the next three paragraphs.

One of the inventors, Dr. Sit, L. Qi and K.H. Sit, Molecular Cell Biology Research Comm., 23, 319-327, 2000, showed that a number of house keeping genes have canonical CpG islands at 5'- promoter region, which are critical in regulation of vital intermediary metabolism and cell structure, whose loss or alteration is central to cell death . House keeping genes such as the gene controlling energy production in glycolysis, tricarboxylic acid cycle (TCA cycle;essential in ATP production), respiratory

electron transport chain. Gene for pyruvate dehydrogenease , whose down regulation causes cell acidification . Basal histone genes, H2A.X , which regulates nucleosome assembly. In this publication it was shown that critical differential gene activity is caused by CpG specific modulation. Such CpG specific modulation was also shown earlier to regulate gene function without changing the informational contents of the genetic code, Razin , A., and Riggs, A.D., Science, 210,604-610, 1980; Falls, J.G., Pulford, D.F., Wylie, A.A., Jirtle, R, Am. J. Patho., 154, 635-647,1999; Jones, P.A., Trends in Genetic, 15, 4310-435, 1999.

It was shown by several authors, that unmethylated CpG islands in the 5'-promoter and intronic sites of transcriptionally active genes are concentrated in the euchromatin domain , which are also susceptible to nucleases , Feil, R. and Khosla, S. Trends Genet., 15, 34-37, 1999; Ng. H.H. and Bird, A., Curr. Opin. Genet. Dev., 9, 158-153, 1999, Bird, A., Trend Genet., 11, 94-100, 1995. It was also shown that CpG methylation confers nuclease resistance to such genes.

Another publication by Dr. Sit, L. Qi and K.H. Sit, Molecular Cell Biology Research Comm., 23, 319-327, 2000, where detailed analysis of a number of the house keeping genes was carried out. The genetic regulation of glycolysis, the tricarboxylic acid cycle (TCA, citrc acid or Krebs cycle) and respiratory electron transport chain involving glucose catabolism and energy requirement of cell. Puruvate dehydrogenase gene regulation was shown to have essential role in this path. And loss of such regulation leads to mitochondrial disfunction.

CpG oligonucleotides selected from such House keeping gene pool which have CpG islands in the promoter region were selected in our earlier studies; L. Qi and K.H. Sit, Molecular Cell Biology Research Comm., 3, 319-327, 2000, and L. Qi and K.H. Sit, Molecular Cell Biology Research Comm., 3, 33-41, 2000. It has been shown in the cited

publications that in mammals caspase dependent and independent pathways are responsible for cell death or apoptosis. In our studies cited in publications cited above, it was shown that CpG ODN block apoptosis. It was further demonstrated that by reversing the CpG motif, viz., GpC or methylation of cytidine, i.e., 5-methylCpG motifs are ineffective in preventing such apoptosis.

CONCLUSION

Applicants believe that by amending the majority of the claims to method claims and amending the product into narrow claims resulting strictly from the application of the disclosed novel method, they have addressed the concerns expressed in the Office Action of 2/26/08.

A Notice of Allowance is respectfully requested. The Examiner is requested to kindly contact the undersigned representative if this communication does not place the case in condition for allowance.

Respectfully submitted,
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